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## Men with coronary artery disease have lower levels of androgens than men with normal coronary angiograms

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**Aims** High androgen levels are presumed by many to explain the male predisposition to coronary artery disease. However, natural androgens inhibit male atherosclerosis<sup>[1]</sup>. Our aim was to determine whether levels of androgens differ between men with and without coronary artery disease.

**Methods and Results** Ninety male subjects (60 with positive, and 30 with negative coronary angiograms) were recruited. Early morning, fasting blood samples were taken from each patient and free, total and bioavailable testosterone, sex hormone binding globulin, oestradiol, and lipids were measured. Bioavailable testosterone was assayed using a modified technique. Free androgen index was calculated. Men with coronary artery disease had significantly lower levels of free testosterone (mean (standard deviation)); 47.95 (13.77) vs 59.87 (26.05) pmol.l<sup>-1</sup>,  $P=0.027$ ), bioavailable testosterone; 2.55 (0.77) vs 3.26 (1.18) nmol.l<sup>-1</sup>,  $P=0.005$  and free androgen index; 37.8 (10.4) vs 48.47

(18.3),  $P=0.005$ , than controls. After controlling for differences in age and body mass index the differences in free androgen index and bioavailable testosterone remained statistically significant ( $P=0.008$  and  $P=0.013$ , respectively).

**Conclusion** Men with coronary artery disease have significantly lower levels of androgens than normal controls, challenging the preconception that physiologically high levels of androgens in men account for their increased relative risk for coronary artery disease.

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**Key Words:** Androgens, gender, coronary artery disease, sex hormones.

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### Introduction

Men are twice as likely to die from coronary heart disease as women<sup>[2]</sup>, a discrepancy which remains unexplained. Pre-menopausal women, who have high levels of endogenous oestrogens appear to be protected from coronary heart disease<sup>[3]</sup>, and post-menopausal women gain benefit from exogenous oestrogens<sup>[4]</sup>. It may be logical to assume that high levels of androgens in men are detrimental to the male cardiovascular system. However, it has recently been clearly demonstrated that replacement of natural androgens inhibits atheroma

formation in castrate male animals<sup>[1]</sup>. There is also increasing evidence in the literature to show that low levels of androgens are associated with adverse cardiovascular risk factors including an atherogenic lipid profile<sup>[5]</sup>, systolic and diastolic hypertension<sup>[6]</sup>, obesity<sup>[7]</sup>, insulin resistance<sup>[8]</sup>, and raised fibrinogen in humans<sup>[9]</sup>.

Studies as early as the 1940s have described a consistent improvement in both anginal symptoms and ischaemia on electrocardiograms in men treated with testosterone preparations<sup>[10]</sup>. More recently, acute intravenous administration of testosterone has been demonstrated to have a marked antiischaemic effect in men with coronary artery disease<sup>[11]</sup>. Testosterone has also been shown to act as a vasodilator in both the coronary and systemic circulations in animal models in-vitro<sup>[12]</sup>, and to augment coronary blood flow in humans in vivo<sup>[13]</sup>.

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Despite the weight of evidence linking low levels of androgens with risk factors for coronary heart disease and changes in coronary blood flow, a number of prospective epidemiological studies have failed to show a relationship between baseline testosterone levels, and the subsequent development of clinically apparent coronary artery disease<sup>[14–16]</sup>. These studies may have been confounded by a number of factors, including failure to control adequately for subclinical coronary artery disease, thereby introducing heterogeneity between the cases and controls, and inadequate assessment of testosterone levels, which is subject to a number of difficulties. Testosterone has both diurnal and circannual variation, making sampling time important. There is also large inter-individual variability making the definition of a truly 'normal' range difficult. Moreover, much of the hormone (68%) circulates in its inactive form tightly bound to sex hormone binding globulin. The bioavailable fraction circulates either free (1–3%) in the circulation or loosely bound to albumin (30%)<sup>[17]</sup>. Previously, researchers have relied on measuring total (and in one study, free)<sup>[14]</sup> testosterone alone, leaving the highly variable and biologically active weakly bound fraction unquantified.

This study was performed to determine whether the sex hormone profile in a group of male patients with coronary artery disease was different to a similar group of men with no coronary artery disease.

We have been careful to avoid the two major potential confounders discussed above, controlling for subclinical coronary artery disease by using controls with proven normal coronary arteries, and by measuring bioavailable testosterone, rather than just free testosterone and total testosterone, and calculating free androgen index, to provide a more accurate assessment of androgen status.

## Methods

### Study design

This case control study was performed from the outpatient population of a large teaching hospital and was approved by the local ethics committee. Levels of bioavailable, free, and total testosterone, free androgen index, oestradiol, luteinising hormone, follicle stimulating hormone, sex hormone binding globulin, total cholesterol, triglycerides, and high density lipoprotein, were compared between a group of men with typical findings of occlusive coronary disease at angiography and a group of men with normal coronary angiograms.

### Subjects

Ninety male patients aged between 33 and 79 years were recruited. Sixty patients had a diagnosis of ischaemic heart disease based upon the finding of >75% occlusion of at least one major coronary artery at coronary

angiography. Thirty control patients with normal coronary angiograms were recruited; 19 of these had valvular heart disease, seven had atypical chest pain, three had congenital anomalies, and one had unexplained dyspnoea. Patients were excluded if they had a myocardial infarction or other major illness within the preceding 3 months, had any past history of hypogonadism, or were taking any medications known to affect sex hormone levels, for example, antiandrogen treatment for prostatic carcinoma.

### Evaluation

Informed, written consent was obtained from all participants. Baseline data including age and history of risk factors for coronary artery disease were recorded. Early morning blood samples (between 0830–0930h) were taken immediately prior to coronary angiography, centrifuged and frozen at  $-20$  degrees Celsius for subsequent analysis. Height and weight were measured and body mass index was calculated by the formula;  $\text{weight(kg)/height(m)}^2$ .

### Laboratory measurements

Percentage bioavailable testosterone was assayed using an adaptation of the method of Tremblay and Dube<sup>[18]</sup>, where  $^3\text{H}$ -labelled testosterone radioactive tracer was measured in the supernatant fraction following ammonium sulphate precipitation of sex hormone binding globulin. The concentration of bioavailable testosterone was then calculated from the percentage of the total steroid. Inter-assay variation was less than 8% throughout the range of the assay. Serum free and total testosterone were measured by radio-immuno-assay (Coat-A-Count, Diagnostic Products Corporation, U.K.), with inter-assay coefficient of variation 5.5% at  $16.3 \text{ pmol.l}^{-1}$  and 3.4% at  $147 \text{ pmol.l}^{-1}$ , and 11.2% at  $3.6 \text{ nmol.l}^{-1}$  and 6.1% at  $13.1 \text{ nmol.l}^{-1}$  respectively. Oestradiol and sex hormone binding globulin were measured by automated enzyme-immuno-assay (Immulate, Diagnostic Products Corporation, U.S.A.). Inter-assay coefficient of variation for oestradiol was 16.7% at  $242 \text{ pmol.l}^{-1}$ , 9.1% at  $2420 \text{ pmol.l}^{-1}$  and 10% at  $5381 \text{ pmol.l}^{-1}$ , and for sex hormone binding globulin was 10.6% at  $5.2 \text{ nmol.l}^{-1}$  and 8.9% at  $73 \text{ nmol.l}^{-1}$ . Luteinising hormone and follicle stimulating hormone were measured using an automated micro-particle-immuno-assay (Abbott AxSYM). Inter-assay coefficient of variation was 8.0% at  $4.5 \text{ Iu.l}^{-1}$ , 6.3% at  $17.1 \text{ Iu.l}^{-1}$ , 12.0% at  $41.7 \text{ Iu.l}^{-1}$  and 6.3% at  $8.9 \text{ Iu.l}^{-1}$ , 7.7% at  $19.4 \text{ Iu.l}^{-1}$  and 6.8% at  $41.7 \text{ Iu.l}^{-1}$  respectively. Lipids were measured using an enzymatic colorimetric test (Olympus, U.K.). Inter-assay coefficients of variation were as follows: Cholesterol; 1.5% at  $3.3 \text{ mmol.l}^{-1}$ , 1.8% at  $6.95 \text{ mmol.l}^{-1}$ , triglycerides; 4.3% at  $1.38 \text{ mmol.l}^{-1}$ , 3.4% at

**Table 1** Baseline characteristics in cases vs controls

	Cases (n=60)	Controls (n=30)	P=
Age (years)*	61 (51–79)	57.8 (33–75)	0.144
Body mass index (kg . m <sup>2</sup> )*	28.4 (22–39.1)	26.4 (22.8–34.2)	0.011
Diabetes	5	1	0.659
Hypertension	25	9	0.282
Smoking	11	4	0.549
Hyperlipidaemia	37	3	<0.001
Statin therapy	40	1	<0.001
Cerebrovascular disease	1	4	0.041

\*Mean (range), *P* value calculated using two-tailed t-test.  
All other *P* values calculated using Pearsons Chi-squared test, or  
Fisher's exact test as appropriate.

2.9 mmol . l<sup>-1</sup>, high density lipoprotein; 7.1% at  
1.05 mmol . l<sup>-1</sup> and 3.7% at 2.05 mmol . l<sup>-1</sup>. Free  
androgen index was calculated using the formula; total  
testosterone/sex hormone binding globulin × 100.

### Statistical methods

Data were analysed using SPSS for Windows 95 computer package. Categorical baseline data displayed in Table 1 were analysed using the chi-squared test or Fisher's exact test where any cell had an expected count

of less than 5. Raw numerical data, displayed in Table 2, comparing cases and controls were analysed using t-tests for independent samples assuming unequal variance. Comparisons between patients with one-, two- and three-vessel disease were made using one way analysis of variance (ANOVA). Adjusted mean differences in hormone levels were calculated using multiple linear regression in which the dependent variables were free androgen index, total testosterone, free testosterone and bioavailable testosterone, and the independent variables entered were age, body mass index and case/control status. No other independent variables were found to be significantly related to hormone levels. The data were inspected visually and the assumption of parallel lines for the relationship between the dependent variable and age or body mass index was satisfied. Thus the values quoted in Table 3 are the coefficients for the difference between cases and controls after adjustment for age and body mass index. All results are expressed as mean (standard deviation) unless otherwise stated. Statistical significance was accepted when *P* ≤ 0.05.

### Results

Baseline characteristics of the two groups are displayed in Table 1. Cases were slightly older, and had higher body mass indices than our control group. As expected, cases had a higher incidence of risk factors for coronary heart disease than controls, although this was only

**Table 2** Results of hormone and lipid analyses in cases and controls (raw data)

	Cases n=60	Controls n=30	P=
Free testosterone (NR 37.4–138.7 pmol . l <sup>-1</sup> )	47.9 (13.77)	59.9 (26.05)	0.027
Total testosterone (NR 7.5–37.0 nmol . l <sup>-1</sup> )	13.3 (4.1)	15.3 (5.59)	0.086
Bioavailable testosterone (NR >2.5 nmol . l <sup>-1</sup> )	2.55 (0.77)	3.26 (1.18)	0.005
Free androgen index (NR 18–50 units)	37.8 (10.39)	48.5 (18.3)	0.005
Oestradiol (NR <150 pmol . l <sup>-1</sup> )	71.8 (33.29)	98.4 (41.63)	0.004
Sex hormone binding globulin (NR 15–75 nmol . l <sup>-1</sup> )	36.4 (11.86)	34.35(13.97)	0.505
Luteinising hormone (NR 1.3–9.1 Iu . l <sup>-1</sup> )	4.25 (2.22)	3.81 (1.89)	0.327
Follicle stimulating hormone (NR 1.7–12.6 Iu . l <sup>-1</sup> )	6.69 (4.58)	5.47 (3.03)	0.134
Total cholesterol (mmol . l <sup>-1</sup> )	5.2 (0.91)	6.0 (1.25)	0.003
High density lipoprotein cholesterol (NR 1.0–1.5 mmol . l <sup>-1</sup> )	0.94 (0.23)	1.01 (0.26)	0.202
Triglycerides (NR 0.5–2.2 mmol . l <sup>-1</sup> )	1.99 (1.09)	1.93 (1.10)	0.809

NR = normal range.  
All values expressed as mean (standard deviation).  
*P*-values stated calculated by independent samples t-testing.

**Table 3** Mean differences in hormone levels in controls versus cases after adjusting for age and body mass index

	Adjusted difference between controls and cases	P=
Free testosterone (pmol . l <sup>-1</sup> )	- 7.318 ( - 15.26 to +0.620)	0.07
Bioavailable testosterone (nmol . l <sup>-1</sup> )	- 0.497 ( - 0.885 to - 0.108)	0.013
Total testosterone (nmol . l <sup>-1</sup> )	- 1.164 ( - 3.28 to +0.952)	0.277
Androgen index (units)	- 7.912 ( - 13.748 to - 2.077)	0.008

Values shown are the mean (95% confidence interval) difference between cases and controls after adjusting for age and body mass index in the regression analysis.

statistically significant for statin therapy and history of hyperlipidaemia. There was a higher incidence of history of cerebrovascular disease (stroke or transient ischaemic attack), amongst the control group.

On examination of the raw data, men with proven coronary artery disease had significantly lower levels of both free and bioavailable testosterone, and free androgen index than normal controls (see Table 2). Levels of total testosterone were also lower in the group with coronary artery disease, although this difference did not reach statistical significance. Total cholesterol level was significantly lower in cases than in controls, a reflection of the higher proportion of cases receiving statin therapy (see Table 1). Oestradiol levels were also significantly lower in the cases than in controls. This result should however be interpreted with caution as the accuracy of oestradiol assays at the low levels seen in males is poor (see methods section).

Hormone levels were compared in cases with one (n=5), two (n=11) or three (n=44) vessel disease, showing no significant differences associated with increasing severity of coronary disease.

Testosterone levels fall with age<sup>[19]</sup>, and are inversely related to body mass index<sup>[7]</sup>. As there were differences in both age and body mass index between our cases and controls, the mean difference in hormone levels between cases and controls was examined after correcting for age and body mass index using multiple linear regression. These results are shown in Table 3. Following correction for age and body mass index, levels of bioavailable testosterone, and free androgen index remain significantly lower in the cases than in controls. Levels of free and total testosterone are also lower in cases than controls, although this relationship fails to meet statistical significance.

## Discussion

In this study, men with proven coronary artery disease were found to have significantly lower serum levels of androgens, compared to men with normal coronary angiograms.

Are the low levels of testosterone seen in these men a cause, a consequence or merely an epiphenomenon of their coronary artery disease?

If hypotestosteronaemia occurred as a consequence of coronary artery disease itself, it may be expected that patients with more severe disease would have lower levels of testosterone than those with only single vessel disease. Our study shows no such stepwise changes in any of the modes of testosterone assayed. It is also unlikely that the low levels of testosterone seen in this study are merely a consequence of general ill health, as 17 of our controls had valvular heart disease, with its associated morbidity, severe enough to warrant referral for surgical intervention.

The low testosterone levels found in the patients in this study may represent merely an epiphenomenon. In case-control studies such as this, there is potential for bias due to inadequate adjustment for confounding variables. Inevitably, cases with coronary artery disease will have very different risk factor profiles from those without coronary artery disease, as demonstrated in Table 1. We chose men with proven normal coronary arteries as our control group to avoid including men with subclinical coronary artery disease as controls. However, the population of men found to have normal coronary arteries at coronary angiography remains markedly atypical of the general population with a high incidence of severe valvular and congenital heart disease.

In this study our cases had higher body mass indices than our controls. Obesity may be implicated as a cause of hypotestosteronaemia via two separate mechanisms. Low levels of testosterone may be expected in men with high body mass index because of increased conversion in body fat and muscle of androstenedione to oestrone, by the enzyme aromatase<sup>[20]</sup>. However, despite a significantly higher body mass index, men with coronary artery disease in our series had significantly lower levels of oestradiol than our normal controls, suggesting that the reduction in testosterone is a primary phenomenon and not related to increased peripheral conversion to oestrogens. Again this result should be interpreted with caution because of the poor accuracy of oestradiol assays at low concentrations. Obesity may also act as an inhibitor of the hypothalamic-pituitary-gonadal axis, possibly via agents such as leptin<sup>[21]</sup>. However, measurements of cumulated gonadotrophin levels did not show any significant differences between the cases and controls. Some workers have argued that although total testosterone levels are low in obese men, a compensatory fall in sex hormone binding globulin results in normalization of free testosterone levels<sup>[20]</sup>. Our data do not support this observation.

It is also notable that significantly more of our cases had a history of hyperlipidaemia and statin therapy than our controls. This high prevalence of statin therapy is reflected in the fact that cases actually had lower levels of total cholesterol than our controls. As cholesterol is the immediate precursor for steroid synthesis, it may follow that the reduction in cholesterol associated with

statin therapy may be the cause of the demonstrated reductions in hormone levels. However, it has previously been shown that statin therapy, with an associated significant reduction in cholesterol levels, has no effect on testicular steroids or steroidogenesis<sup>[22]</sup>.

We have discussed the possibility that differences in identified cardiovascular risk factors between our two groups may have been responsible for the significantly lower levels of hormones amongst our cases, and have therefore attempted to statistically correct for these potential confounders. Despite this, significant differences remain between the two groups, implying that the low androgen status demonstrated in these men may be a risk factor for the development of coronary atherosclerosis.

This study shows that there is a positive association between low serum androgen levels and the presence of coronary artery disease. These data challenge the pre-conceived notion that the levels of androgens seen in the reproductive adult male are detrimental to the male cardiovascular system. Whether low androgen status is a predisposing factor for coronary atherogenesis, a secondary phenomenon to the disease process, or an epiphenomenon is not clear. Waning levels of androgens in elderly men are important in the development of osteoporosis<sup>[23]</sup>, sexual dysfunction<sup>[24]</sup>, and symptoms such as depression and fatigue<sup>[25]</sup>. Androgen replacement therapy may prove useful in certain elderly male patients, and this work suggests that this would, at least, confer no increased cardiovascular risk, and may be of cardiovascular benefit.

Our findings provide support for more extensive evaluation of the role of androgens in the development of cardiovascular disease in men.

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